



- (51) **International Patent Classification:**
C12N 1/21 (2006.01) *C12N 9/02* (2006.01)
- (21) **International Application Number:**
PCT/US2014/013712
- (22) **International Filing Date:**
30 January 2014 (30.01.2014)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/758,441 30 January 2013 (30.01.2013) US
61/828,675 29 May 2013 (29.05.2013) US
- (71) **Applicant: LANZATECH NEW ZEALAND LIMITED**
[NZ/NZ]; 24 Balfour Road, Parnell, 1052 Auckland (NZ).
- (72) **Inventor; and**
- (71) **Applicant (for US only): MUELLER, Alexander**
[US/NZ]; 24 Balfour Road, Parnell, 1052 Auckland (NZ).
- (72) **Inventor: KOEPKE, Michael;** 24 Balfour Road, Parnell, 1052 Auckland (NZ).
- (74) **Agent: MOLINARO, Frank, S;** LanzaTech New Zealand Limited, 8045 Lamon Avenue, Suite 400, Skokie, IL 60077 (US).
- (81) **Designated States (unless otherwise indicated, for every kind of national protection available):** AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,

BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

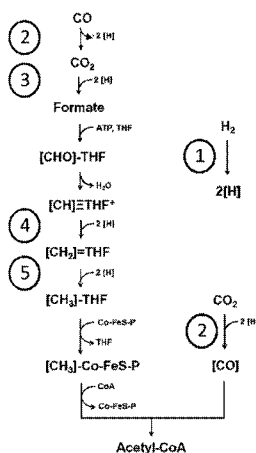
- (84) **Designated States (unless otherwise indicated, for every kind of regional protection available):** ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
 - before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
 - with sequence listing part of description (Rule 5.2(a))
- (88) **Date of publication of the international search report:**
5 February 2015

(54) **Title:** RECOMBINANT MICROORGANISMS COMPRISING NADPH DEPENDENT ENZYMES AND METHODS OF PRODUCTION THEREOF

Enzyme	Substrate	Specific activity (U/mg)
① Hydrogenase	H ₂ + MV _{ox}	275
	H ₂ + Fd _{ox}	<0.01
	H ₂ + NAD ⁺	0.05
	H ₂ + NADP ⁺	0.2
	H ₂ + Fd _{ox} + NAD ⁺	<0.01
② CO dehydrogenase	H ₂ + Fd _{ox} + NADP ⁺	0.68
	CO + MV _{ox}	1.4
	CO + Fd _{ox}	2.7
	CO + NAD ⁺	0.1
③ Formate dehydrogenase/ Formate-Hydrogen lyase	CO + NADP ⁺	0.2
	Formate + MV _{ox}	10
	Formate + Fd _{ox}	<0.01
	Formate + NAD ⁺	0.03
	Formate + NADP ⁺	0.2
	Formate + Fd _{ox} + NAD ⁺	0.07
④ Methylene-THF dehydrogenase	Formate + Fd _{ox} + NADP ⁺	1.10
	Formate + 2H ⁺	2.4
	Methylene-THF + Fd _{ox}	<0.01
⑤ Methylene-THF reductase	Methylene-THF + NAD ⁺	<0.01
	Methylene-THF + NADP ⁺	1.12
	Methyl-THF + BV _{ox}	1.93
	Methylene-THF + NADPH	<0.01
	Methylene-THF + NADH	<0.01



(57) **Abstract:** The invention provides a recombinant carboxydophilic Clostridia microorganism with increased overall utilization of NADPH relative to a parent microorganism. Further, the invention provides a method of producing a recombinant carboxydophilic Clostridia microorganism which exhibits increased NADPH utilization relative to a parental microorganism. In particular, the invention relates to increasing the overall utilization of NADPH in a recombinant carboxydophilic Clostridia microorganism in order to increase the production of at least one fermentation product by the microorganism.

A. CLASSIFICATION OF SUBJECT MATTER**C12N 1/21(2006.01)i, C12N 9/02(2006.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N 1/21; C12P 19/04; C12M 1/00; C12N 5/10; C12P 7/16; C12P 7/02; C12M 3/00; C12N 9/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & keywords: NADPH-dependent enzyme, NADP, carboxydutrophic Clostridia, fermentation, cofactor, formate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012-088071 A2 (LEE, J. W.) 28 June 2012 See paragraphs [0366]-[0371]; claims 1-20 ; figures 4-10 and 13-17.	1-2, 13-16, 26-29
A		3-12, 17-25, 30-46
X	WANG, S. et al., `NADP+ reduction with reduced ferredoxin and NADP+ reduction with NADH are coupled via an electron-bifurcating enzyme complex in Clostridium kluyveri`, Journal of Bacteriology, 30 July 2010, Vol. 192, No. 19, pp. 5115-5123. See abstract; page 5116, left column and page 5118, left column; table 1; figures 1 and 4-6.	36, 38
X	US 2010-0151543 A1 (REEVES, A.) 17 June 2010 See abstract; claims 9-23.	1, 13-15, 26-29
X	US 2009-0191599 A1 (DEVROE, E. J. et al.) 30 July 2009 See abstract; claims 1-12, 34-35 and 40-42.	1, 13-15, 26-29
X	WO 2009-111513 A1 (JOULE BIOTECHNOLOGIES INC.) 11 September 2009 See abstract; claims 1, 10-11 and 47-56.	1, 13-15, 26-29



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

21 November 2014 (21.11.2014)

Date of mailing of the international search report

21 November 2014 (21.11.2014)

Name and mailing address of the ISA/KR

International Application Division
Korean Intellectual Property Office
189 Cheongsu-ro, Seo-gu, Daejeon Metropolitan City, 302-701,
Republic of Korea

Facsimile No. +82-42-472-7140

Authorized officer

HEO, Joo Hyung

Telephone No. +82-42-481-8150



INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2014/013712

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012-088071 A2	28/06/2012	EP 2655599 A2 EP 2655599 A4 JP 2014-501109 A US 2011-0177571 A1 US 2013-0344553 A1 WO 2012-088071 A3	30/10/2013 30/04/2014 20/01/2014 21/07/2011 26/12/2013 06/12/2012
US 2010-0151543 A1	17/06/2010	US 8039239 B2	18/10/2011
US 2009-0191599 A1	30/07/2009	AU 2008-323673 A1 CA 2704227 A1 CA 2728285 A1 CA 2740400 A1 CN 102015995 A CN 102216463 A CN 102216463 B CN 103725717 A EP 2217695 A2 EP 2285948 A1 EP 2285948 B1 EP 2327769 A1 EP 2327769 B1 EP 2344652 A1 EP 2615164 A1 EP 2706111 A1 JP 2011-516029 A JP 2014-087360 A US 2009-0203070 A1 US 2011-0008861 A1 US 2011-0124073 A1 US 2011-0151531 A1 US 7785861 B2 US 7968321 B1 US 7981647 B2 WO 2009-036095 A1 WO 2009-062190 A2 WO 2009-062190 A3 WO 2009-111513 A1 WO 2010-006312 A2 WO 2010-006312 A3 WO 2010-017245 A1 WO 2010-036951 A2 WO 2010-036951 A3 WO 2010-044960 A1	14/05/2009 14/05/2009 11/09/2009 22/04/2010 13/04/2011 12/10/2011 16/10/2013 16/04/2014 18/08/2010 23/02/2011 08/01/2014 01/06/2011 27/03/2013 20/07/2011 17/07/2013 12/03/2014 26/05/2011 15/05/2014 13/08/2009 13/01/2011 26/05/2011 23/06/2011 31/08/2010 28/06/2011 19/07/2011 19/03/2009 14/05/2009 17/09/2009 11/09/2009 14/01/2010 27/05/2010 11/02/2010 01/04/2010 24/06/2010 22/04/2010
WO 2009-111513 A1	11/09/2009	AU 2008-323673 A1 CA 2704227 A1 CA 2728285 A1 CA 2740400 A1	14/05/2009 14/05/2009 11/09/2009 22/04/2010

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2014/013712

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		CN 102015995 A	13/04/2011
		CN 102216463 A	12/10/2011
		CN 102216463 B	16/10/2013
		CN 103725717 A	16/04/2014
		EP 2217695 A2	18/08/2010
		EP 2285948 A1	23/02/2011
		EP 2285948 A4	29/02/2012
		EP 2285948 B1	08/01/2014
		EP 2327769 A1	01/06/2011
		EP 2327769 B1	27/03/2013
		EP 2344652 A1	20/07/2011
		EP 2344652 A4	21/03/2012
		EP 2615164 A1	17/07/2013
		EP 2706111 A1	12/03/2014
		IL 207951 D0	30/12/2010
		IL 212363 D0	30/06/2011
		JP 2011-516029 A	26/05/2011
		JP 2014-087360 A	15/05/2014
		MX 2011004049 A	27/09/2011
		MX 301000 B	05/07/2012
		US 2009-0191599 A1	30/07/2009
		US 2009-0203070 A1	13/08/2009
		US 2011-0008861 A1	13/01/2011
		US 2011-0124073 A1	26/05/2011
		US 2011-0151531 A1	23/06/2011
		US 2011-0262975 A1	27/10/2011
		US 2011-0281335 A1	17/11/2011
		US 2012-0015427 A1	19/01/2012
		US 2012-0122193 A1	17/05/2012
		US 2012-0149092 A1	14/06/2012
		US 2013-0252300 A1	26/09/2013
		US 7785861 B2	31/08/2010
		US 7968321 B1	28/06/2011
		US 7981647 B2	19/07/2011
		US 8048666 B1	01/11/2011
		US 8216816 B2	10/07/2012
		US 8227237 B2	24/07/2012
		US 8465954 B2	18/06/2013
		WO 2009-036095 A1	19/03/2009
		WO 2009-062190 A2	14/05/2009
		WO 2009-062190 A3	17/09/2009
		WO 2010-006312 A2	14/01/2010
		WO 2010-006312 A3	27/05/2010
		WO 2010-017245 A1	11/02/2010
		WO 2010-036951 A2	01/04/2010
		WO 2010-036951 A3	24/06/2010
		WO 2010-044960 A1	22/04/2010